“Occurrence of Virulent Strains of Helicobacter Pylori and its Association with Endoscopic Findings in a Tertiary Centre Teaching Hospital in Chennai”

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BRANCH- IV

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UNIVERSITY CHENNAI,

TAMILNADU

CERTIFICATE

This is to certify that this dissertation entitled **"Occurrence of virulent strains of helicobacter pylori and its association with endoscopic findings in a tertiary centre teaching hospital in chennai"** submitted by **Dr.AnandVadivel** to the Faculty of Medical Gastroenterology, The TamilnaduDr.MGR Medical University, Guindy, Chennai-600032, in partial fulfillment of the requirement for the award of DM Degree, Branch IV (Medical Gastroenterology) is a bonafide work carried out by him under my direct supervision and guidance, during the academic year 2011-2014.

Prof. Dr. P. Ganesh, M.D., D.M
Professor and HOD/ Guide
Department of Medical Gastroenterology,
(DDHD@GPH, Annanagar),
Kilpauk Medical College
Chennai

Dr. P. Ramakrishnan, M.D., D.L.O
Dean,
Kilpauk Medical College,
Chennai

DECLARATION

I **Dr. V. Anand** declare that I carried out this work on **"Occurrence of virulent strains of helicobacter pylori and its association with endoscopic findings in a tertiary centre teaching hospital in chennai"** at the Department of Medical Gastroenterology, Govt.Peripheral Hospital and Kilpauk Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, diploma to any university, board either in India or abroad.

This is submitted to the TamilnaduDr.M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulation for the D.M. Degree examination in Medical Gastroenterology.

Govt. Kilpauk Medical College Dr. V. Anand

Chennai.

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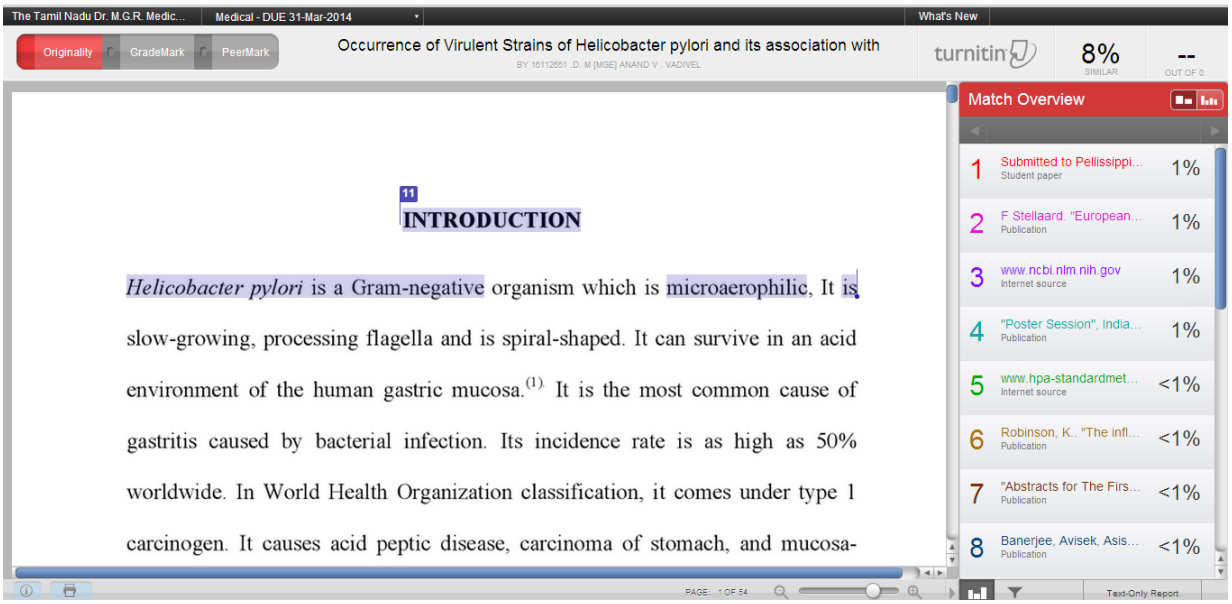
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INTRODUCTION
Helicobacter pylori is a Gram-negative organism which is microaerophilic, It is slow-growing, processing flagella and is spiral-shaped. It can survive in an acid environment of the human gastric mucosa.(1). It is the most common cause of gastritis caused by bacterial infection. Its incidence rate is as high as 50% worldwide. In World Health Organization classification, it comes under type 1 carcinogen. It causes acid peptic disease, carcinoma of stomach, and mucosa-
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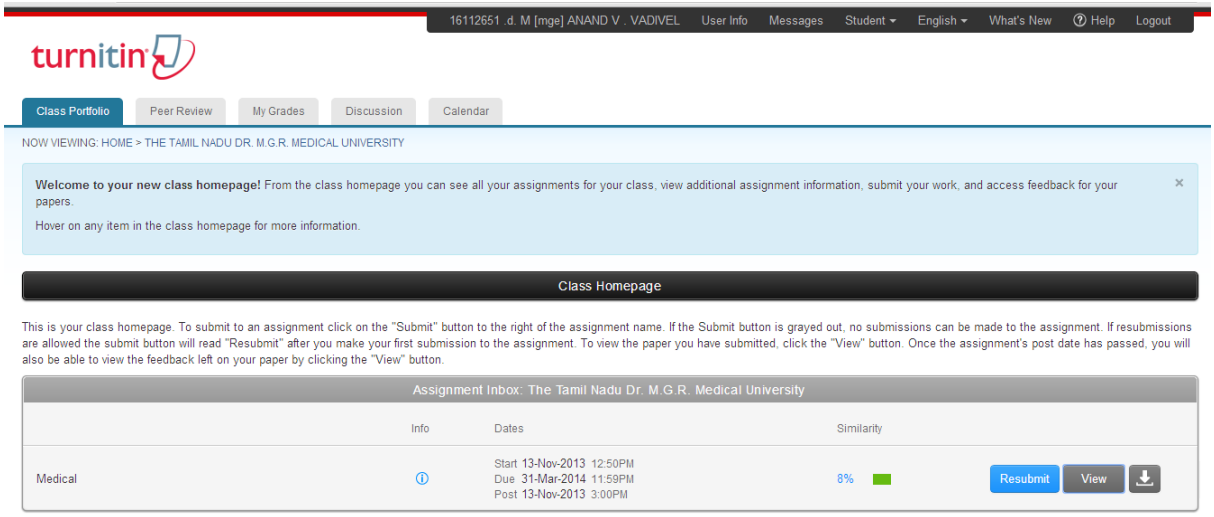
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INTRODUCTION

INTRODUCTION

Helicobacter pylori is a Gram-negative organism which ismicroaerophilic, It is slow-growing, processing flagella and is spiral-shaped.It can survive in an acid environment of the human gastric mucosa.[1]. It is the most common cause of gastritis caused by bacterial infection. Its incidence rate is as high as 50% worldwide. In World Health Organization classification, it comes under type 1 carcinogen. It causes acid peptic disease, carcinoma of stomach, and mucosa-associated lymphoid tissue lymphomas(MALT).[2]

Once infection is acquired in humans, it persiststhroughout the life, till completion of antimicrobial therapy.In developing countriesinfectionprevalence ishigh (70–90%) compared to 25–50 % in developed countries. In India*H. pylori* prevalence is very high,in that 70–90% were with duodenal ulcer, and 50–80% were asymptomatic adults[3].

H. pylori colonization, histologicallyreveals chronic gastritis mostly, remaining shows peptic ulcers and gastric adenocarcinomas. Gastric cancer is one of the three major causes of cancer related deathsworldwide , leading to considerable socioeconomic costs. chemotherapy for gastric adenocarcinoma does not have improved survival benefit. However, it is possible to prevent gastric cancer by eradication of the infection.

Upper GI endoscope was done, the mucosal morphology of the gastric corpus was classified into 4 patterns and correlated with histology and rapid urease test[7,8] . In invasive diagnostic methods, molecular biological methods play a major role for the detecting the species. *H. pylori* DNAhave beendetected in gastric biopsy specimens by PCR assays. It can also detected in stool samples, saliva and in dental plaque.PCR assays detects the urease gene, the 16S ribosomal RNA genesequences and adhesin genesequences of *H. pylori*[9,10].

Resistance to antimicrobials is increasing, necessitating an altered regimen.The treatment of *H. pylori* infection combines two to three antimicrobials, and PPI to increasetheeradication rate and to decrease the antibiotic resistance .The antibiotic-resistanceto *H. pylori* strains is increasing, which hinders the eradication of infection. The resistancefor metronidazoleis10-90% and for clarithromycinis0 to15% [3]. The anti *H pylori*eradication regimen should be selected based on anti- microbial sensitivity with fewer adverse effects. Thus, culture and drug sensitivity test is a very useful tool for selecting appropriate drug regime.

Thyagarajan et al. (2003) in their large multicentric study report an overall resistance of around 78% to metronidazole,45% to clarithromycin and 33% to amoxycillin. Multiple resistance was seen in 43% isolates. The resistance toMetronidazole was higher in Chennai (88%) and Hyderabad (100%).Resistance toCiprofloxacin and tetracycline was less than 4% [11].

Several virulence genes from *H. pylori* have been identified and their role in pathogenecity has been documented. These include the cytotoxin associated gene (Cag A), vacuolatingcytotoxin associated gene (vac A), epithelial gene (ice A), blood group antigen binding adhesion gene (Bab A), and duodenal ulcer-promoting gene A (dupA). Cag A plays a role in duodenal ulcer, gastric cancer and gastro mucosal atrophy and Vac A in peptic ulceration. Mukhopadhyay et al. (2000) have reported 80 to 90% of Calcutta strains carry the cag pathogenicity island and vacAs1 alleles of the vacA are highly toxic [4].

Mishra et al.(2002) havereported that all isolates from Lucknow in their study, to possess vacA and about 96.2% of isolates from Lucknow were cagA positive[5]

Udhayakumar et al. (2009) have reported 96% of their isolates from Chennai to be CagA positive and 60% were Vac m2 subtype [6].

We carried out this study in our centre to identify the mucosal patterns of Helicobacter pylori associated gastritis in the gastric corpus using video upper G.I endoscopy and study the frequency and relevance of association of virulence factors and clinical presentation among H. pylori infected individuals.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Chronology / Historical perspectives

Year :	Event
1886	Jaworski's- Describe the spiral organisms in gastric washingsof humans.
1893	G. Bizzazero. - Spiral organisms confirmed in animals.
1900	Salomon - Spiral bacteria infecting dogs can be transmitted to mice
1906	Kreinitz - Infecting stomach was confirmed .
1910	Schwartz - "No acid no ulcer"
1924	Luck and Seth - Urease in the human stomach(natural)
1940	Freedberg and Baron - Spirochetes in autopsied .
1953	Dintzis and Hastings - Relationship between urease and bacteria
1967	Susumu Ito - Campylobacter-like organisms in gastric epithelialcell.
1982	Marshall and Warren - Accidental culturing of H. pylori done.
1985	Marshall - Self-induced infection[12] .
1992	Covacci et al. - Sequence the CagA gene cause duodenal ulcers. (first virulence factor)
1994	Parsonnet et al. - Association between lymphomas GIT and*H. pylori*
2002	Maastricht Consensus Report, - "test-and-treat" strategy for *H.pylori*
2005	Warren and Marshall - Awarded the Nobel Prize in Physiology or Medicine for their work on *H. pylori* and PUD

Bacteriology

*Helicobacter pylori*is agram negative helical bacteria about 3 µm long with a diameter of 0.5µm.It is a microaerophilici.e. requiring very low concentrations of oxygen. It has hydrogenase activity by which it gets energy by molecular hydrogen oxidation. Other enzymes produced by these bacteria are urease, oxidase andcatalase. Like other Gram-negative bacteria,its outer-membrane ismade up of lipopolysaccharideand phospholipids.

FIGURE 1: *Helicobacter pylori.*

India Scenario

In a developing country like Indiawith vast rural population, majority of people belongs to low socio-economic group ,where the prevalence of *H. pylori*is 80 percent. The Clinical manifestation of *H. pylori* infection in India is peptic ulcer disease, particularly duodenal ulcer disease. When compared to gastric ulcers the ratio is 8:1 and 30:1. Singh et al. “calculated the point prevalence of active peptic ulcer disease as 3% with a lifetime prevalence of 9 per cent”. The outcome from a*H. pylori* infection is predicated based on the pattern of gastritis[13].In antral predominant gastritis,gastric acid secretion is normal but inhibitory function of

antrum is decreased which leads to uncontrolled acid secretion and forms duodenal ulcer. In pan-gastritis,acidsecretion is decreased resulting in atrophic gastritis which is more pronefor gastric cancer[14].

FIGURE 2 : Global prevalence of *H. pylori*.

Dietis an important environmental factor that plays amajor role in the *H. pylori*associated gastric diseases. Tropical dietconsists of plenty of fruits and vegetables which cause non-atrophic gastritis and duodenal ulcer.[15,13]. Seasonal diets,predominantly the salt preserved foods lack fresh fruits and vegetableswhich leads topan-gastritis and gastric ulcers. In south India duodenal ulcer prevalence is very high due to these environmental factors are play a major role.

Gastric cancer incidence rate is2 to 57 persons per 1 lakh [16].The population of India according to [CENSUS 2011] is approximately 1.2 billion people. The prevalence of *H. pylori*was 60 %, that means more than 726 million people would be infected with *H. pylori*[17]. "The estimated prevalence of duodenal ulcers

in India is 3 per cent and means that at least 18 million people could need anti-*H. pylori* therapy (approximately 50,000 per day if treated over one year)".

Direct transmission

Transmission of bacteria from person to person occur by fecal- oral, oral - oral, and gastro - oral[18].

1) Oral-oral transmission

*H. pylori*was previously thought as oral microbes, [19], although it is not prevalent among the dentist and dental workers[20,21]. Recent studies stated that persons exposed todiarrhoea caused by *H. pylori* is a high risk for new infection [22],

2) Gastro-oral transmission

Since*H. pylori* commonly inhabits the human stomach, it wassuggested that gastric juicerefluxis thecause for direct gastro-oral route .[23]Several studies reported that even the vomitus of infected subjects cause transmission.[24].Among infected siblings^{13}C urea breath test holds good for detection of organisms [25].

3) Feco-oral transmission :It is unlikely to occur via feco- oral transmission since bile has bactericidal effect.[18]

II. Indirect transmission

The sources and the reservoir of infection were investigated from food, animals, and water sources based on its morphology and DNA study.But no evidence suggested them as primary vehicles of transmission.

H. pylori reservoirs

Hypothesis	**Evidence**
Food Unhygienicfood	Positive correlation
Raw vegetables	likelihood of infection
Sheep and cow milk	
Animals	
Most animal species act as *H. pylori* reservoirs	Sheep/cat /rhesus monkeys /houseflies /cockroaches
Animal-to-human	Dore et al. stated that *H pylori* is a zoonotic, occupational infection animals.
Water	likelihood of infection

Genetic and Environmental Determinants

Age

The prevalence increases with age in developing and developed countries . The prevalence of infection was high in adults .

Genetic andEthnic factors

Many studies reported marked differences in H. pylori seroprevalence among various ethnic and racial groups [26]. It was suggested that genetic factors also influence *H. pylori*infection,based on study in monozygotic and dizygotic twins.[27].

Gender

H. pylori infection is highly prevalent among men .[28]

Interfamilial relations

Several studies reported as spread of infection among families in children is from adults.[29].The interfamilial spreading is mainly caused by mother-to-child transmission and spouse-to-spouse transmission [30].

Socioeconomic factors

H. pylori infection is highly prevalent among lower socioeconomic groups. The socioeconomic status includessocial class, income and also includes standard of living, sanitation, urbanization, and educational level [11].

Crowding index (Density of living)

H. pylori infection is highly prevalent among densely crowded areas. The risk factors include sharing a bed, crowding of houses and increasing household contact.[32],

Factors and pathogenesis

Microbial virulent factors

Bacterial virulence factors are associated with inflammation of gastric mucosa.[33]

CYTOTOXIN

Leunk et al. first described the vacuolatingcytotoxin (VacA) in eukaryotic cells.[32] The cytotoxin produced by the organism is responsible for peptic ulcer disease. The VacA protein has abiological activity which increases at acidicpH.VacA isactivated by acid pH and is highly associated with mucosal damage.

Atherton et al stated that "there are two divergent regions in vacA. One is in the second half of the signal sequence (s1a/s1b and s2) and the other is in the mid-part of the gene (m1 and m2)".

The s1/m1 strains are highly virulent allelic than s1/m2strains, gastric epithelial damage high among m1 strains [34] and mucosal infiltrates with lymphocyte and neutrophils is usually seen among s1 strains [34].

Cytotoxin associated gene A

cagA is commonly encountered gene of *H pylori*. Ithas a molecular weight of (120–140 kDa) and is immune-dominant, seen in 60% strains.[35].

CagA strains results in increased immune responses at mucosal level and severe gastric inflammation . Thus CagA positive strains has astrong association withatrophic gastritis,acidpeptic disease and gastric cancer.

A 40 kilobase pathogenicity island (cag PAI) includes cagAand other 30 genes. The gene products from cag PAI assess the response of epithelial chemokine using isogenic mutant strains. [36]. CagA serves as a marker for virulent organism Different genes in the cag PAI has a varied response in epithelial chemokine. Cag PAI genes codes for protein which has a secretory function in host epithelium .[37].

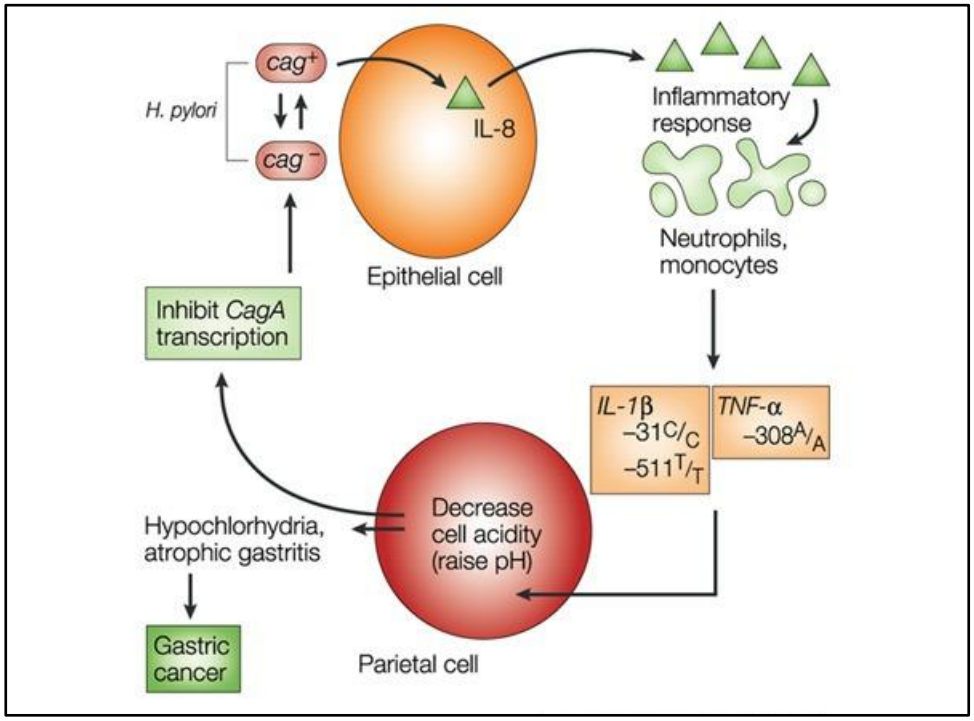


FIGURE 3- Equilibrium of interactions between H. pylori and its host.

Lipopolysaccharide

The *H pylori*lipopolysaccharide[LPS] has lowimmunological activity and low biological activity due to its lipid Acomponentwhich is in a phosphorylated state and is responsible for chronic inflammation andautoimmune responses in the stomach at mucosal level.Similarity exists between LPS O-specific antigen and host antigen –LewisX,LewisY blood group antigens[38]. This molecular mimicry plays a role in H+,K+ proton pump mechanism where the anti- LewisY antibodies produced by *H pylori* binds to Lewis Y epitopeleading to atrophic gastritis. Lewis antigens more frequently expressed on cagA positive strains.[39] .

Immune mediated damage:

The inflammatory responseby host immune systemalso inducesdamage of gastric mucosa. Histologically, it is characterised by infiltration of lymphocytes,plasma cells, monocytes,and neutrophils, into gastric mucosa.[40].

Neutrophil activation

Activated neutrophils is the first inflammatory response to *H pylori*. HP-NAP (Neutrophil activated protein) and LPS(lipopolysaccharides) via superoxide stimulate neutrophils .Epithelium secretes chemokines like IL-8 and regulated

cytokines such as TNF-α and IL-1. Increased expression of ICAM-1 and VCAM-1 cause neutrophil endothelial adhesion and extravasation.

T cell activation:

Chronic gastritis frequently secrete interferon (IFN) –γ by H pylori specific CD4+ T cell clones. *H pylori* infection also stimulate IL-12 production from peripherial blood leucocytesand induce Th1 response. Peptic ulcer disease is induced by Th1 response.

Complement activation

Infection activates the classic pathway of complement and leads to neutrophil chemotaxis and epithelial damage.Berstad and colleagues states that activation of both complement pathways.

Features of Gastritis The Acute Phase **:** Initially subclinical.

Ingestion of *H. pylori*

⬇

Mucous layerPenetration andmultiplication occur in surface epithelial cells.

⬇

Cellular exfoliation, mucin depletion, and regeneration .

⬇

Infiltration of polymorphs intofoveolar and surface epithelium

⬇

Edemaof lamina propria

⬇

Hypochlorhydria[41].

This acute infectionspontaneously resolve in minor population particularly in children. In most of the patients infection fails to resolve and gradually develop into chronic active gastritis in 4 weeks

Active Chronic Gastritis

Acute inflammatory responseaugmented by Lymphocytes and plasma cells in the mucosa leads to production of antibodies andcytokines as second-line response involves production of lymphocytes and plasma cells. Gastric mucosa containing lymphoid tissue develops into gastric marginal zone (B-cell) lymphoma called MALToma.

Atrophy

Gastric Atrophy is defined as "loss of glandular tissue from repeated mucosal injury due to long-standing infection". It leads to erosion or ulceration of the mucosa, prolonged inflammatory process causing fibrous replacement. Mucous metaplasia is defined as parietal cells are replaced by mucous cells.

*H. pylori*prevalenceisinversely related to glandular atrophy due to

1. Colonizes only in gastric epithelium;

2. Survives only in acidic environment .[42].

Intestinal Metaplasia(IM)

It isreplaced by gastric epithelium into intestinal epithelium and is always associated with abnormal growth stimulation [43].IM is most commonly associated with elderly, commonly seen in the antrum, associated with gastric ulcer and bile reflux.[44].

Antral Predominant Gastritis

H. pylori in the presence of urea and acidic environment

↓

To maintainproton motive force (PMF) in periplasmic membrane.

↓

Cytoplasmic urease acts on urea entering the bacterium to produce ammonia.

↓

Ammoniapreserve the PMF by maintaing a pH of 6.2 in the periplasmic space[45].

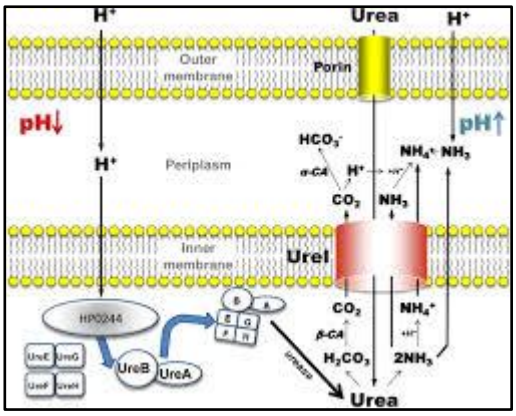


FIGURE 5 -Model of the mechanism of action of UreI and HP0244

Corpus Gastritis

A corpus gastritis is seen in small group of patients.Corpus-predominant gastritis has low acid output due to release of IL-1from parietal cell which is acid-inhibitory cytokines .

Pathogenesis of Duodenal Ulceration :

Gastric Metaplasia

Hyper-gastrinemia and increased sensitivity to gastrin present in duodenal ulcer causes unneutralized acid in the duodenal bulbresulting in compensatory gastric metaplasia[mucus-secreting cells][(46).]

Active Chronic Duodenitis

*H. pylori*gets attached to gastric epithelium,it is site where polymorphs migrate into epithelium.In duodenum has gastric metaplasia associate with polymorph response.Identification of *H. pylori*organism is difficult in duodenal biopsies. The prevalence rateofduodenalulcerandgastric metaplasia is diagnosed by histology, culture, and rapid urease tests.

Ulceration

Wyatt et al.emphasized the relationship betweenactive chronic duodenitis,*H. pylori*gastritis,acid-induced gastritis cause metaplasia.[47].

*H. pylori*spread from the stomach to duodenum ⟶ Induodenum acid-induced metaplasia⟶ Chronic inflammation⟶ duodenal ulceration.

Gastric Ulceration

Gastric ulceration occurs due to an intense inflammatory response secondary to H.pylori infection at the antral corpus transitional zone. This intense inflammatory response due to *H. pylori* at the acid-gradient transitional zone is based on thelocal environment andoptimal pH, orinduction of stress proteins resulting in release of inflammatory products.

Gastric cancer

Gastric adenocarcinoma caused by *H. pylori* infection is associated with other factors like host factors,genetic factors,strain of bacteria, duration and environmental factors.

In Correa pathway cancer,Due to DNA alterations occurred by *H. pylori* infection and inflammation.It leads to recruitment and bone marrow-derived cells engraftment .Altered epithelial cell proliferation and apoptosiswhich leads to atrophy. *H. pylori* with nitrate reductase activity, leads to adenocarcinoma. Corpus-predominant atrophy, or the loss of specialized glandular cell are the first step in cancer .

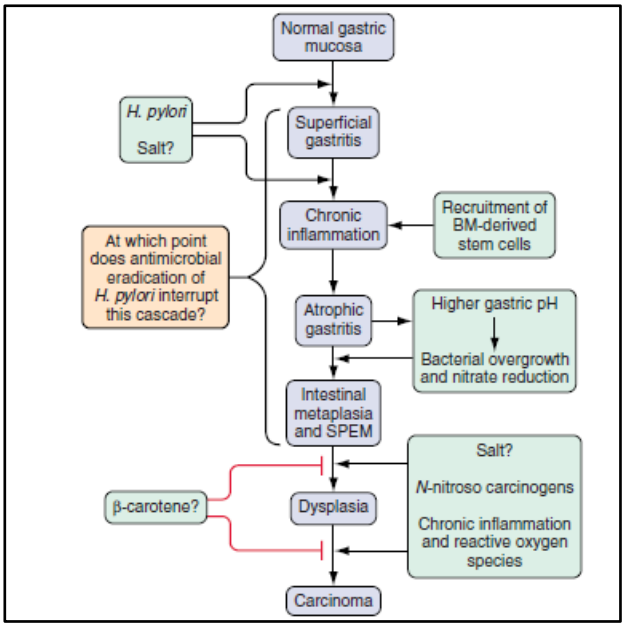


FIGURE 6-Model representing the role of H. pylori and other factors in gastric carcinogenesis, based on the cascade proposed by Correa et al.

Role of Bacterial strains

FlaA and FlaBproteins - 1)Motility toward epithelial cells of the stomach

2) ECM and mucus altered and cause decrease Viscosity.

UreA/UreB complex - Buffering of stomach and survives.

Hop proteins	- Adhesion of bacteria to epithelium .
BabA gene	- Adhere to fucosylated Lewis B blood group antigen .
babA2 gene	- Binds to epithelial cells and associated with adenocarcinoma.
TFSS	- Secreting system (injects cag)
cag pathogenicity island	- Severe inflammation atrophic gastritis and adenocarcinoma.
vacA gene	- T cell activation inhibitor.
cage	-Altered apoptosis and inflammatory response in host.

Non-steroidal AntiInflammatory Drugs (NSAIDs) with *H. pylori*

A study conducted on *H. pylori* association with NSAIDS based onmucosal erosions, haemorrhage and ulcers following I week of NSAIDS intake.[48] It revealed no association between *H pylori* and normal population.

Extraintestinal manifestations:

Ischaemic heart disease & cerebrovascular disease

H pylori may influence IHD by cross mimicry between vascular endothelium, such as heat shock proteins[HSP], leading to pro-coagulablestate due to infection[49].

Functional Vascular Disorders

H. pylori infection is associated with idiopathic migraine andRaynaud's phenomenon.[50]

Immunological diseases

Several studies reported,After eradication of *H pylori* patients with autoimmune diseases (HSP, Sjögren's syndrome, and idiopathic thrombocytopenia)[51]. Some cases of extragastricMALT type of lymphoma improved after treatment for *H pylori* infection.[52].

Skin diseases

H. pylori cytotoxic strains cause mast cell degranulation which triggersdevelopment of urticaria.[53]*H. pylori* infection strongly associated withAcne rosacea and alopecia areata[54].

Liver and biliary tract

Elevated blood concentrations of ammonia decreases on treating *H. pylori*.[55]. A recent study established a relation between *H pylori*in bile samples with

CagAprotein and aminopeptidase N, which induces cholesterol aggregation. But association with *H. pylori* and cholelithiasis is not known.[56].

Others complications

H. pylori infection associated with sideropenic anaemia [57].sudden infant death and decrease growth rate in children[58].

INVESTIGATIONS:

Invasive techniqueslike tissue biopsy was followed earlier. With advancement in diagnostic field, non invasive tests like molecular biology has become popular, but not ideal for routine use[59]

Gastric endoscopic pictures of H.pylori infection (Taiwan classification)

Morphology of the gastric mucosa was classified into 4 types, observed with upper GI endoscopy.

Type 1 -"Cleft-like appearance extending along the longitudinal axis of gastric body".

Type 2 -"Red dots arranged uniformly"

Type 3 - "The mosaic pattern without a focal area of hyperemia."

Type 4 -"The mosaic pattern with a focal area of hyperemia ."[60]

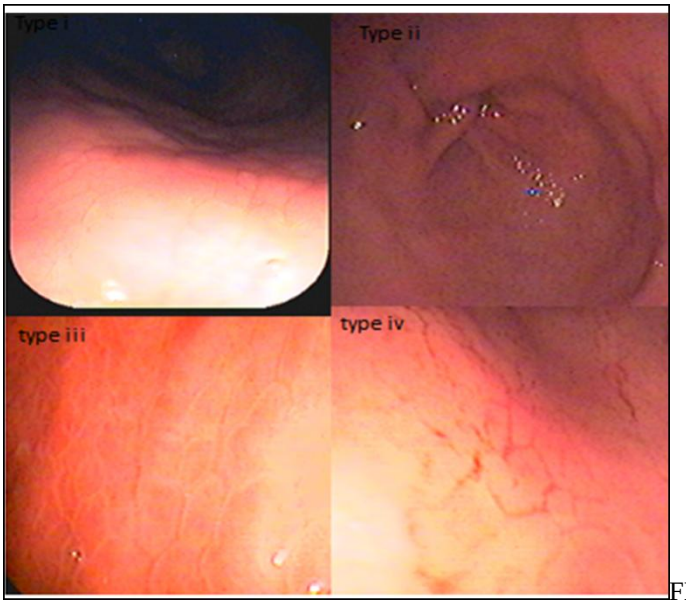


FIGURE 7- Mucosal patterns in endoscopy.

In gastric ulcer or gastric atrophy , biopsy sampling was taken from the nearby non-atrophic mucosa. Gastric biopsy is the specimen forculture and sensitivity.[61]

Invasive techniques:[62]

- Histopathological examination
- Culture and sensitivity
- Rapid urease test(RUT)
- Polymerase chain reaction (PCR)

Culture

*H.pylori*are slow growing and fastidious organism. It needs specialmedia and growth environment hence it is difficult to culture. Culture enables performance

of antimicrobial susceptibility testing. It helps to know about treatment efficacy, and re-infection identification. Though the sensitivity rate is 92% , In refractory disease, for prediction of treatment outcome it has limited role.

Histology

Though *H pylori*infestationcan be detected with routine hematoxylin and eosin staining, it is not sensitive, hence requires special stains such asGiemsa, silver, or immune stainswhich are specific. Histologic examination is the gold standard with reported sensitivity and specificity 95% and 98%, respectively. Histology and culture does not provide rapid diagnosis.[63]

Biopsy urease test

Rapid urease test is highly sensitive and cost effective. It is combined with culture or histology. Commercial kits are highly accurate but expensive.

Also called CLO test (Campylobacter-like organism test), performed to detect urease producing organism. The bacterial enzyme urease converts ureato ammonia and CO2, resulting in colour change of the broth due to increase in pH.(phenol red acts as a pH indicator.)

Urea is produced during decarboxylation of the amino acid arginine in the urea cycle. It is water soluble hence it released from human body when present in excess.

H. pylori is more efficient in hydrolyzing urea than other enteric bacteria, hence it is named as"rapid urease-positive" organism.

Polymerase Chain Reaction (PCR)

PCR – To detect *H. pylori* in various samples.

Indications :

1. Research

2.Difficulty in culturing.

3. For epidemiologic studies by testing stool or drinking water

4.Tissue - “real time” antibiotic resistance.

Non-invasive techniques

These methods are comparatively inexpensive and avoid hazels of endoscopy,

• Serology (ELISA)

• Urea breath tests (UBTs)

•Stool antigen tests

Serology

Serology is primarily done by ELISA IgGand IgA. [64]. IgGis the primary detection modality. The sensitivity is 90% -100%and specificity is 76% - 96 %.

Serologyhas a high negative predictive value and a poorpositive predictive value ina low prevalence area. Serology has a limited value in detection of eradication of *H. pylori*due to the persistence of immunoglobulins**.**

Urea breath tests (UBTs)

It is currently the most commonly used non – invasive technique to identify H.pyloriinfection.[65]Urea is labelled with either ^{14}C or ^{13}C. The specificity and sensitivity is about 95%, thus making false-positive results a rarity. Sensitivity of the test can be improved by avoiding antibiotics for 4 weeks and anti- secretory drugs for 1 week before breath test. UBTs can be used for assessment of eradication therapy .UBT is not accurate in post gastrectomy status.

Stool Antigen Tests

The *H. pylori* stool antigen (HpSA) test is one of the newer diagnostic modality to diagnose of H. pylori[66].It is a non-invasive enzymeimmunoassay with a sensitivity and specificity of around 95% with the advantage to confirm eradication.

Current recommendations for testing

1. For an initial diagnosis of *H. pylori* aUBT or stool antigen assay is opted because of they are non-invasive and their ability to detect active infection.

2. Serology excludes*H. pylori* infection, and for active infections positive serology results requires a confirmatory test.

3.Biopsy should be done in patients undergoing a diagnostic endoscopy for a suspected ulcer or MALT lymphoma. If histopathology is not required Biopsy urease testing can be done, in patients not on anti-secretory or antibiotics.

4. An UBT or astool antigen test can be done after 4 to 6 weeks of therapy if clinically indicated for confirmation of successful eradication of infection.

5. In a complicated ulcer disease or other mucosal abnormality, after 4-6 weeks of treatment endoscopy should be done for follow up.

Treatment

Indications for anti-*H. Pylori* regimen

The Canadian *H. pylori* Consensus Conference recommend that it is ideal to treat all*H. pylori*-positive patients. Even if another aetiology is suspected along with which documentation of eradication of the infection would be appropriate.[67]

Recommended primary therapiesinclude: clarithromycin or metronidazole, amoxicillin, Proton pump inhibitor (clarithromycin-based triple therapy) for 14 days or 10 to 14 days of treatment with metronidazole, bismuth, tetracycline , PPI or H2RA, (bismuth quadruple therapy).Intention-to-treat (ITT) analytical studies have shown an eradication rates hover around 70–80% [68].Trials have documented that amoxicillin or metronidazole have equal efficacy in a

clarithromycin-based triple therapy so metronidazole can be substituted in place of amoxicillin in patients allergic to penicillin. Bismuth quadruple therapy is an alternative for penicillin allergy patients who had been previously treated with a macrolide based regimen .

An alternative to triple and quadruple therapy is the sequential therapy which is amoxicillin and Proton Pump Inhibitor for five days followed by , PPI tinidazole and clarithromycin for fivedays. Sequential therapy has been found to be superior in clarithromycin resistant areas.

Culture and sensitivity are rarely performed due to the expense lack of availability, the usefulness of such testing in providing alternative regimens in patients who have failed the primary treatment modality.

Bismuth based quadruple therapyis“salvage” therapy .This salvage regimen is widely available, inexpensive, and relatively effective.

Newer regimens

Triple therapy -based onLevofloxacin(levofloxacin, and amoxicillin PPI).Rifabutin, is an alternative to clarithromycin (eradication rate- 38% to 91%.).

AIM:

AIM:

1. To identify the mucosal patterns of *H. pylori* associated gastritis using video upper G.I endoscopy.

2. To study the frequency of H. pylori virulence factors and their correlation with the clinical presentation.

3. To determine the association of virulence genes of H. pylori to that of the mucosal changes on endoscopy and histopathology.

MATERIALS AND METHODS

MATERIALS AND METHODS

This is a hospital based cross-sectional studyconducted inthe Department of Digestive Health and Diseases(DDHD), Government peripheral hospital, Chennai from December 2012 to January 2014.150 patients with symptoms of dyspepsia were included in the study. The study was initiated after obtaining approval by the ethics committee of Kilpauk medical college. Patients were be enrolled following the inclusion / exclusion criteria provided below. Written informed consent was obtained from all participating subjects in regional language (Tamil). Privacy and confidentiality was ensured.

Inclusion criteria

1. Uninvestigated dyspepsia
2. Active gastric ulcer or duodenal ulcer
3. Gastric MALT-lymphoma (low grade)

Exclusion criteria

1. Structural cause for dyspepsia (hiatus hernia, GERD)
2. Metabolic cause for dyspepsia (uremia, ischemic heart disease, biliary colic, pancreatitis)
3. Recent use of proton pump inhibitor, antibiotics, bismuth-containing compounds
4. Patients who refused consent.

All patients were investigated for CBC, Blood glucose, blood urea,serumcreatinine, ECG, Ultrasound abdomen , blood grouping & typing and Gastroscopy

Six biopsy samples were taken directly from the suspicious sites (antrum or corpus).

Two samples were sent for histological analysis at Department of Pathology , Kilpauk Medical College

Histopathological examination - Specimens were placed in 10% formalin solution and routinely processed.

Two samples for a rapid urease test.

Two samples for culture and antibiotic susceptibility testing, Genomic DNA extraction.

Each antral and corpus specimens for culture were transported to the NIE laboratory in a suitable transport medium i.e. sterile isotonic saline if transported within 6 h or in sterile BHIB at 40C in a vaccine carrier / thermcol box with frozen gel packs.

Composition of Urea Broth:(Rustigian and Stuart, 1941*) – Ref: Baltimore Biological Laboratories, Maryland, USA

Ref: Myer's and Koshi's- CMC Vellore "Manual of Diagnostic Procedures in Medical Microbiology and Immunology/ Serology"

Urea	-	2.0gm
Monopotassium Phosphate (KH2PO4)	-	0.91 gm
Disodium Phosphate (Na2HPO4)	-	0.05 gm
Yeast Extract	-	0.01 gm
Phenol Red	-	0.001 gm
Distilled water	-	100ml

The ingredients was dissolved in distilled H_2O and pH as adjusted to 6.8. The base mediums was sterilized by autoclaving at 115^0C for 10minutes and then add filter sterilized urea solution to the autoclaved base.The pH was adjusted to 6.8. 1ml of broth was distributed in small sterilized vials.

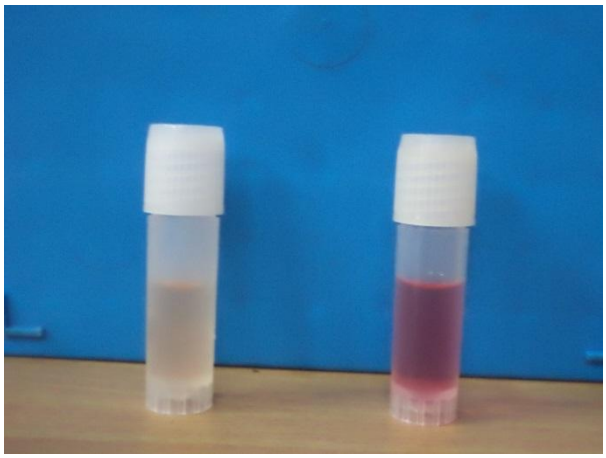


FIGURE 8- Rapid Urease Broth negative (L) and Positive (R)reactions for *H. pylori*.

Transport medium

Endoscopy specimens were transported to the NIE laboratory in a suitable transport medium i.e. sterile isotonic saline if transported within 6 h or in sterile BHIB at 40C in a vaccine carrier / thermcol box with frozen gel packs .

Brain Heart Infusion Broth :

Beef Heart Infusion Form - 250 gm/ liter

Calf Brain Infusion Form - 200 gm/ liter

Dextrose - 2 gm / liter

Proteose peptone - 10 gm / liter

NaCl- 5 gm / liter

Disodium Phosphate - 2.50 gm / liter

37gms of above mentioned compounds were dissolved completely in 1000ml of distilled water, pre-autoclaved at 121^0C for 15 minutes adjust pH to 7.4. 1ml of broth was distributed in small sterilized vials.

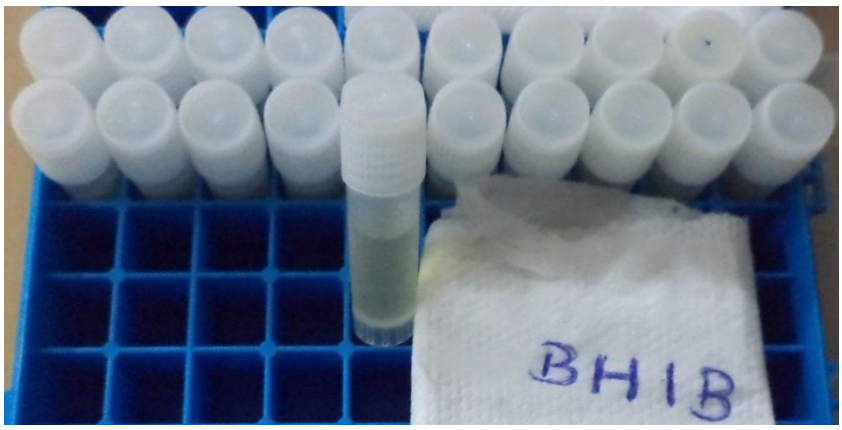


FIGURE 9- Transport Medium - Brain Heart Infusion Broth.

Histopathology

One portion of the biopsy material was sent for staining with hematoxylin and eosin for histopathogical examination at Kilpauk Medical College. Scoring for gastritis and gastritis activity, from mild to severe, was done based on the level of infiltrating lymphocytes and neutrophils, respectively. Grading in bacterial density was be performed by observing the bacterial count present on gastric epithelia. The presence of large bacterial clumps was considered as severe, whereas a single or small group of two to three organisms was graded from mild to moderate. Other important parameters including atrophy, intestinal metaplasia, and chronic or active gastritis if any, was documented.

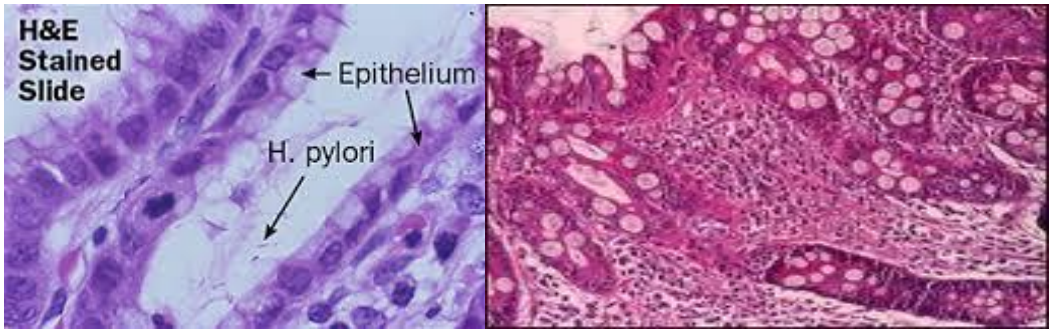


FIGURE 9- *H. pylori* in antrumFIGURE 10- Intestinal Metaplasia

Culture

Culture of tissue homogenate wasattempted on 5-7% sheep blood agar containing Skirrow's supplement (Oxoid). The plates were incubated at 370^{o}C under microaerophilic conditions (10% CO_2) for upto 10 days. It was planned to perform antibiotic susceptibility testing for any positive culture .

Genomic DNA extraction from biopsy specimen

DNA from each biopsy specimen was extracted using the genomic DNA purification kit (Himedia), according to the manufacturer's instructions. Extracted DNA was stored at -20^{o}C until PCR analysis.PCR assays for glmM gene and virulence genes (vacA, cagA) was carried out as per standard protocols. Analysis for the human β-globulin housekeeping gene will be carried out by PCR to assess the quality of extracted DNA.

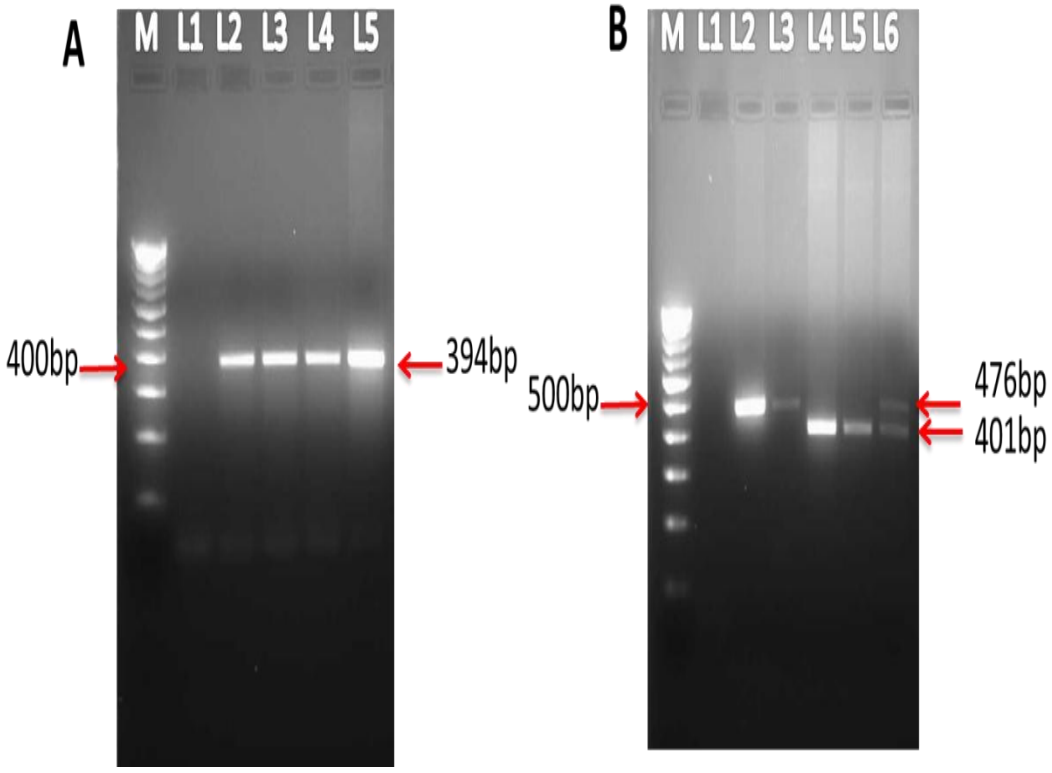


Figure -11

Fig: (A). Amplification of *cagA* gene(394 bp), M=100bp ladder, Lane 1 Negative control and Lane 2 to 4 biopsy samples
(B). Amplification of *vacA* gene (Allele m1 (401 bp) and Allele m2 (476bp), M=100bp ladder, Lane 1 Negative control, Lane 2 to 3 Allele m2, Lane 4 to 5 Allele m1 and Lane 6 Allele m1 m2

All endoscopies were performed by experienced endoscopist.

Ethical committee approval was obtained from Kilpauk medical college before starting the study.

Written informed consent was obtained from all participating subjects in regional language (Tamil). Privacy was ensured.

Statistical analysis was done by statistical analysis software SPSS(version 19.0)

RESULTS

RESULTS

A total of 147 patients were included for the study based on inclusion criteria.

Age distribution

Among 147 patients, 50% of cases were from the age group 21-39 years followed by middle age(29%).Out of them*H. pylori* positivity (PCR) was seen in 35 patients and 12 patients respectively . Table 1

Age (years)	Frequency (%)
<20	9 (6)
21-39	74 (50)
40-59	40 (29)
>60	22 (15)
Total	**147**

Table1. Age wise distribution of study participants

Gender distribution

Among the 147 patients in our study, 105(71%) were males and 42 (29%) were females (Table:2).Out of them *H. pylori* positivity (PCR) was seen in 42 patients and 17 patients respectively.Table 2

Sex	**Frequency** (%)
Male	105 (71)
Female	42 (29)
Total	**147**

Table2. Gender wise distribution of study participants

Presenting complaints : In our study, abdominal bloat(27%) was the most common symptom, followed by epigastric pain(24%) & postprandial fullness (14%). The less common symptoms were early satiety (5%),atypicalchestpain (4%), & nausea & vomiting(1%).In 15% of patients symptoms were relieved by antacids.

36 patients had epigastric pain out of which 16 patients (44.4%) showed positivity for H. pylori and 39patients had Abdominal bloating out of which 15patients (38.4%) showed positivity for H. pylori *H. pylori* positivity among different clinical symptoms was not statistically significant (P =0.0714).

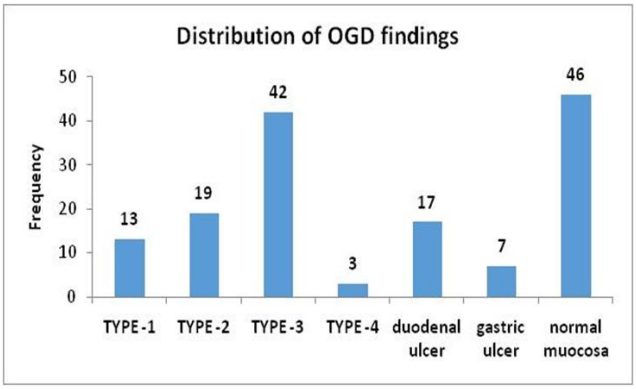
Personal habits : In our study, frequency of alcohol(26%) & smoking(22%) was almost equal. But majority(29%) of them had no risk factors. Among the patients with the above listed personal habits ,occurrence of PCR positivity ismore or less equal varying from 36% to 50% .Though PCR positivity was slightly more in risk group(alcohol,smoking) the finding was not statistically significant (P:0.968).

Past history : Past history was not significant in majority of the patients40%. 28% had diabetes mellitus, followed by Hypertensionin 17% and Drug intake like ASA ,NSAID in 15%.Among patients withdrug intake, 11/22 patients showed PCR positivity. In 41 Diabetic patients 20 had PCR positivity.Though PCR was positive in almost 50% of patients with drug intake and diabetes group, overall it was not statistically significant (P value:0.217)

Endoscopy findings

Among 147 patients, abnormal findings was seen in 69 % and 31% had normal mucosal study. Out of 69% , type 1 & 2 gastritis was seen in 22%, type 3&4 was observed in 31%, duodenal ulcer in 12% and Gastric ulcer in 5% of participants.Figure 12.

Figure 12: Distribution of endoscopic findings



Histopathologicalfeatures : Our study showed 84 %patients had gastritis and 16 % patients had precancerous lesions i.e., atrophic gastritis and intestinal metaplasia and *H. pylori* was seen microscopically in only 2% of participants.Table 3

Table 3: Distribution of histopathological findings

Histopathological Examination (HPE)	Frequency (%)
Chronic non -specific gastritis	83 (56)
Erosive gastritis	24 (16)
Nodular gastritis	14 (10)
Intestinal metaplasia	17 (12)
Atrophic gastritis	6 (4)
H. Pylori	3 (2)
Total	**147**

Percentage of RUT and PCR positivity

Among 147 patients, RUT was positive in 46 % and PCR positive in 40% of the patients. Table 4.

Table 4: Results of RUT and PCR analysis

Rapid Urease Test (RUT)	**Frequency** (%)
Positive	68 (46)
Negative	79 (54)
Total	**147**

PCR	**Frequency (%)**
Positive	59 (40)
Negative	88 (60)
Total	**147**

Comparison of RUT with PCR positivity

On comparing RUT with PCR, RUT showed sensitivity of 71.19 % and specificity of 70.45 % , PPV of 61.76% and NPV of 78.48%. Table 5.

Table 5: Comparison of RUT & PCR findings

RUT	PCR		Total
	Positive	Negative	
Positive	42	26	68
Negative	17	62	79
Total	59	88	147

Comparison of endoscopic findings with PCR

In PCR positive group, sensitivity of abnormal endoscopic findings are 69.49% and specificity of 31.82%. PPV & NPV of 40.59%& 60.87% respectively.Table6

Table 6: Comparison of endoscopy findings with PCR

OGD	PCR		Total
	Positive	Negative	
Positive	41	60	101
Negative	18	28	46
Total	59	88	147

Comparison of endoscopy positive findings with PCR

Among abnormal findings, PCR positivity was high in Type 3 & 4 gastritis in comparison to Type 1 & 2, with sensitivity of 84.38% and specificity of 60%. Even in normal mucosa PCR was positive in 39% of patients as we are using conventional endoscopyrather thanmagnification endoscopy .Overall association of endoscopic positivity and PCR is statistically significant (P = 0.003).Table 7

Table7: Endoscopic positivity & PCR results.

OGD	PCR		Total
	Positive	Negative	
Type 1 + Type 2	5	27	32
Type 3 + Type 4	27	18	45
Duodenal ulcer	7	10	17
Gastric ulcer	2	5	7
Normal mucosa	18	28	46
Total	59	88	147

Comparison of endoscopy positive findings with Cag positivity

In endoscopy Cag positive patients were 37,out of which 21 (56.75%) had gastritis including all types.Cag positivity was more in type 3 & 4 gastritis and duodenal ulcer groups. But among gastric ulcer, none was positive. p value was 0.059 .Table 8

Table8 : Comparison of endoscopy positive findings with Cag positivity

OGD FINDINGS	Cag gene		Total
	Positive	Negative	
Type 1 + Type 2	4	28	32
Type 3 + Type 4	17	28	45
Duodenal ulcer	5	12	17
Gastric ulcer	0	7	7
Normal muocosa	11	35	46
Total	37	110	147

Comparison of endoscopy positive findings with Vac positivity

Out of the 53 VacA positive patients, 24(53%) had type 3& 4 gastritis and 5(15%) had type 1&2 gastritis.VacA positivity wasmore common in type 3 & 4 gastritis (s_1m_2>s_1m_1)and duodenal ulcer in comparison with Cag gene.Both s_1m_1 and s_1m_2 were positive in 11%. Table 9.

Table 9 :Comparison of endoscopy positive findings with Vac positivity

OGD findings	Vac genes				Total
	M1+	M2+	M1+M2	Negative	
Type 1 + Type 2	1	3	1	27	32
Type 3 + Type 4	8	14	2	21	45
Duodenal ulcer	2	2	1	12	17
Gastric ulcer	0	1	0	6	7
Normal muocosa	9	7	2	28	46
Total	20	27	6	94	147

Comparison of Histopathological features with Cag gene

Among 37 Cag positive patients,27 patients presented with gastritis and 10patients had precancerous lesions i.e., atrophic gastritis(2/10) and intestinal metaplasia(8/10) . Out of 27 patients who had gastritis, 17 (62%)had Chronic non -specific gastritis, 5(18.8%)hadErosive gastritis , 4(14.8%) had Nodular gastritis and 1 (3.7%) had H.pylori gastritis .Around 72.9% Cag positivepatients were associated with gastritis.

Cag only positive subgroup gastritis was seen in 4(14.8%)of the 27 patients.In the Cag subgroup only one person had a precancerous lesion.

Out of 37 Cag positive patients, 22 patients had both Cag and Vac genepositivity.Table 10

Table 10: Comparison of histopathological features with Cag gene

HPE	Cag A		Total
	Positive	Negative	
Chronic non -specific gastritis	17	66	83
Erosive gastritis	5	19	24
Nodular gastritis	4	10	14
Intestinal metaplasia	8	9	17
Atrophic gastritis	2	4	6
H. pylori	1	2	3
Total	37	110	147

Comparison of Histopathological features with Vac gene

Among 53 Vac positive patients, 20(37.7%) patients had s_1m_1, 27(50.9%) patients hads_1m_2 and both positive were seen in 6(11.4%) patients.

In the VacA only positive sub group, gastritis was seen in 17 (44.7%)patients out of the 38. On subtyping, 6(32.2%) patients had s_1m_1, 9 (52.9%)patients had s_1m_2 and 2(11.7%) persons had both $s_1m_1$and s_1m_2.

In the VacA positive group, precancerous lesions i.e., atrophic gastritis and intestinal metaplasia were seen in 7 patients.On subtyping 6(85.7%) patients had $s_1m_2$and only 1(14.2%) had both s_1m_1 and s_1m_2.

11/17(64.7%) patients with intestinal metaplasia showed VacA positivity , in which 8/11 patients (72 %) were positive for s_1m_2.Table 11.

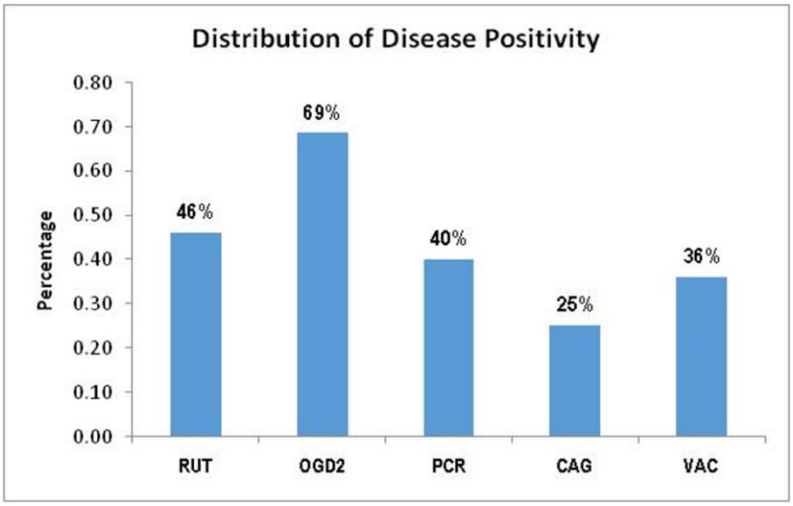
Table 11:Comparison of Histopathological features with Vac gene

HPE findings	Vac gene				Total
	M1+	M2+	M1+M2	Negative	
Chronic non -specific gastritis	8	9	4	62	83
Erosive gastritis	5	5	0	14	24
Nodular gastritis	3	2	0	9	14
Intestinal metaplasia	3	7	1	6	17
Atrophic gastritis	1	3	0	2	6
H. pylori	0	1	1	1	3
Total	20	27	6	94	147

Distribution of disease positivity

Overall, 69 % patients had abnormal endoscopic findings, 46 % had RUT positive and 40 % had PCR positive.Rapid urease test had a sensitivity of 71%,specificity of 70.45 % and cost effective which can be carried out in the endoscopy suite. As the disease positivity of RUT is more or less equal with PCR , patients can be treated based on RUT .Recommended for use in combination with either culture or histology, depending on local facilities. Figure 13

Figure 13:Depictsdistribution of disease positivity :



Test	RUT	OGD	PCR	CAG	VAC
Positive(No s)	68	101	59	37	53
% of Positivity (POS/147) * 100	46	69	40	25	36

DISCUSSION

Helicobacter pylori is a Gram-negative organism which is microaerophilic, spiral shaped, slow-growing with processing flagella.Once infection is acquired in human itpersistsstroughout the life, till completion of antimicrobial therapy. *H. pylori* colonization results mostly in chronic gastritis, followed by peptic ulcers and gastric adenocarcinomas. Investigation of *H. pylori*by Upper GI endoscope reveals the mucosal morphology of the gastric corpus as 4 types of patterns ,duodenal ulcer and gastric ulcer. The invasive diagnostic methods such as Rapid urease test , histopathological examination and molecular biological methods, play a major role for detecting the species. *H. pylori* DNAhave beendetectedin gastric biopsy specimens by PCR assays. Genes are classified as Cytotoxinassociated gene (Cag A), Vacuolatingcytotoxin associated gene (vac A), epithelial gene (ice A), blood group antigen binding adhesion gene (Bab A), and duodenal ulcer-promoting gene A (dupA). Cag A plays a role in duodenal ulcer, gastric cancer and gastro mucosal atrophy and Vac A in peptic ulceration.

The present study identified the mucosal patterns of *Helicobacterpylori*associated gastritis in the gastric corpus using video upper G.I endoscopy and compared with PCR which is confirmatory test .In addition we assessed the frequency of H. pylori virulence factors and their correlation with the clinical presentation and also we evaluated the association of virulence

genes of H. pylori to that of the mucosal changes on endoscopy and histopathology.

Alwahaibi et al. stated that among the 366 patients included in their study, 30.05 % patients were *H. pylori* positive based on histopathology[69]. In another study, Hermann Brenner et al. reported a prevalence of 21 % (94/447) with ^{13}C-urea breath test[30].Study by Leen-Jan Van Doorn et al. showed a positivity of 36.7% out of 493 cases based on PCR.[70] In this study of 147 patients ,59 patients (40%) were positive for *H. pylori* PCR.

Alwahaibi et al. also showed that *H. pylori*infection was more common in the younger age group (34.5%) followed by middle aged persons(30.9%).He further reports that female gender (36.9%) was more prone to develop *H. pylori* infection than the male gender (22.2%) [69]., Kate et al also had similar kind ofresults being that of 63% men and 85% women positive for the infection[71].This study also had a similar age distribution , the age group between 21to 40 years contributed to the majority of the infected individuals in the study group (59%) followed by the middle age group those in-between 41 to 60 years (20%), these results are similar to studies by Alwahaibiand Kate but no gender differences were seen in this study group.

Leo et al. in a meta analysisstates thatepigastric pain had a positive correlation with *H. pylori*[72]. In thisstudy 44.4% with epigastric painand 38.4%

with abdominal bloating showed positivity for H. pylori .Lin et al and Donohoe et alshowed that clinical features had no correlation to*H.* pylori infection .

Hermann Brenner et al showed*H. pylori* had a positive correlation with coffee intake and smoking but had a negative correlation with alcohol consumption [(30)]**.** In this studyH. pylori was detected in 40% of coffee drinkers, 39% of smokers and 36% of alcohol abusers but none had a significant association with the non-substance abusers.

In this study , RUT sensitivity, specificity, PPV, NPV were 71.19 % , 70.45 % , 61.76% and 78.48% respectively which is lower when compared to the study by Lage et al who reported RUT sensitivityof 89.5 % and specificity97%.[(73).] Factors such as in house preparation of RUT medium could have resulted in batch variation.

We attempted to culture initial 40 biopsy samples but none of them grew *H. pylori*. Factors such as delay in transportation of samples to lab, in house media preparation, use of gas packs rather than CO_2 incubator could all have contributed to failure to isolate the organism from samples that were otherwise RUT and PCR positive. Further culturing of remaining samples were not undertaken.

Sheng-Lei Yan et al[7] hypothesized that Type 1 and 2 mucosal patterns on endoscopy were good predictors of *H. pylori*-negative status while Type 3 and

type 4 mucosal patterns were good predictors of *H. pylori* positivity. This study is akin to the above study showing a high PCR positivity in type 3 & 4 gastritis in comparison to type 1 & 2 with a sensitivity of 84.38% and a specificity of 60% . There is not much literature comparing H. *pylori* virulent genes to the mucosal patterns seen on endoscopy. The virulent Cag genes were positive in4 patients of Type 1&2 ,17 patients of Type 3&4 and Vac genes were positive in 5 Type 1&2 ,24 patients ofType 3&4 . 5 patients of Cag genes andVac genes were positive in duodenal ulcer patients . 39% of patients had PCR positivity inspite of a normal mucosa in conventional endoscopy , probably due to early stage of infection .

Kim et al found 91% showed histologic gastritis and 96% had *H. pylori* infection in his study population.[74], This study has demonstrated histologic gastritis in all the individuals which varied from non- specific gastritis to atrophic gastritis but *H. pylori* was found only in 3 patients. The possible reasons could be a sample not from the specified area, inadequate tissue size, defect in fixation, improper staining, thick microtome cutting of sections or excessive inflammatory cells & debris obscuring the organisms.

PCR positivity of glm gene of H. pylori was seen in 59(10.1%) patients of the study population. Meanwhile the incidence of the virulence genes by the way of only Cag positivity was seen in 6(10.3%) patients, only Vac positivity in 22(37.2%) patients and both virulence genes were seen in 31(52.5%)patients.

Among 37 Cag positive ,27 patients had gastritis and 10patients had precancerous lesions .Cag only positive subgroup gastritis was seen in 4of the 27 patients. Out of 37 Cag positive patients, 22 patients had both Cag and Vac genepositivity. 70% Cag positive patients were associated with gastritis.

Previous Vac genotype studies by Fukui et al. showed 81.25% of $s_{1c}m_{1b}$where asOkinav et al. had predominant 70.4%$s_{1c}m_{1b}$.In this study ,among 53 Vac positive patients, 20(37.7%) patients had $s_1m_{1,}$27(50.9%) patients hads_1m_2 and both positive was seen in 6(11.4%) patients Among patients with Vac positivity the subtype s1m2 constituted 50% while s_1m_1 was seen in 37.7% and both were seen in 11.3% of individuals

In the VacA only positive sub group of the study, gastritis was seen in 17 patients out of the 38, and on subtyping, 6 had s_1m_1, 9 had s_1m_2 and 2 persons had both $s_1m_1$and s_1m_2;

In this study,16 patients had intestinal metaplasia and 6 had atrophic gastritis. Out of 12/16 of intestinal metaplassia& 4/6 of atrophic gastritis patients were PCR positive,but in them s1m2 of Vac were predominantly associated with intestinal metaplasia and atrophic gastritis. This shows s1m2 of Vac is highly virulent and considered as precancerous. In contrast toTomohisa Uchida et[75] al which says s1m1 is more virulent and carcinogenic.

SUMMARY

In the study population of 147, 59 patients were positive for *H. pylori*diagnosed by the presence of genetic studies. Out of the 59 patients Type 1 and 2 gastritis were seen in 5 patients , while Type 3 and 4 gastritis were seen in 27 patients thus indicating a more severe gastritis seen in individuals infected with *H. pylori.* Ulcers were seen in 9 patients out of which duodenal ulcer was seen in 7 patients while there were 2 patients with gastric ulcer. There were no mucosal abnormalitiesas observed on conventional endoscope in 18 patients.

PCR positivity of glm gene of H. *pylori* was seen in 59(40%) patients of the study population, meanwhile the incidence of the virulence genes by the way of Cag positivity was seen in 6patients, Vac positivity in 22 patients and both virulence genes were seen in 31 .Among patients with Vac positivity the subtype $s_1m_2$constituted 50% whiles_1m_1 was seen in 37.7% ,both were seen in 11.3% of individuals.

The predominant complaint in the study was abdominal bloating and epigastric pain ,which had a 38.4% and 44.4% correlation with *H. pylori* PCR .

In the VacA only positive sub group of the study, gastritis was seen in 17 patients out of the 38, and on subtyping 6 patients had s_1m_1, 9 patients had s_1m_2 and 2 persons hadboth $s_1m_1$and s_1m_2; while in the Cag only positive subgroup gastritis was seen in 4of the 27 patients.

In the VacA positive group precancerous lesions i.e., atrophic gastritis and intestinal metaplasia were seen in 7 patients on subtyping 6 patients in $s_1m_2$and only one had both s_1m_1 and s_1m_2. In the Cag subgroup only one person had a precancerous lesion.In the subgroup who had both Cag andVacA genes , 22 had gastritis and 9 patients had precancerous lesion.There was significant correlation s1/m2 virulent gene of H. *pylori* to that of intestinal metaplasia and atrophic gastritis.

In this study there was an insignificant difference in between PCR and RUT , thus confirming that RUT even though a cheap test is valuable in establishing the diagnosis of *H. pylori* in an office setting.

CONCLUSION

- Our study identified predominant type 3 and 4 mucosal pattern followed by type 1 and type 2. Also PCR correlates well with type 3 and type 4 pattern.
- Among the prevalence of virulent genes, Vac gene is most common than Cag, with the predominant subtype being s_1m_2.
- There was significant correlation of H. pylori virulent genes s_1m_2 to that of intestinal metaplasia and atrophic gastritis
- In this study disease positivity of RUT is more or less equal with PCR , confirming that RUT is valuable in establishing the diagnosis of *H. pylori* .

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LIMITATIONS :

- Conventionalendoscopy was used rather than amagnification endoscopy which would have been ideal in observing the mucosal pattern.
- Genomic extraction was done only for two genes Cag A and vac A.Other genes like ice A, Bab A anddupA has not been evaluated .
- Lack of workup for other causes of gastritis.

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MASTER CHART

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	DDHD NO	age	SEX	C /F	PAST	personal	RUT	HPE	OGD	PCR	cag	vac
S. No	DDHD NO:	<20yrs-1 21-39-2 40-59-3 >60yrs-4	M=1 F=2	PPF-1;BL-2 ES-3;EB-4 AN-5;HB-6 NV-7;CP-8	DM-1 HT-2 DG-3 NIL-4	AL-1 SM-2 CO-3 SK-4 NO-5	1 = $+_{VE}$ / 2 = $-_{VE}$	1 = CNG 2 = EG 3 = NG 4 = IM 5 = AG 6 = HP	1 = TYPE -1 / 2 = TYPE-2 3 = TYPE-3 4 = TYPE-4 5 = DU / 6 = GU / 7 = N	1=+ve 2 = ve	1=$+_{VE}$ 2= $-_{VE}$	1= M1 $+_{\sout{VE}}$ 2=M2$+_{VE}$ 3=M1+M2$+_{VE}$ 4=-ve
1.	1494/13	3	2	1	1	5	1	1	7	2	2	4
2.	115/13	4	1	2	3	2	2	1	5	1	1	1
3.	186/13	2	1	4	4	3	1	1	7	1	2	2
4.	1857/13	3	1	5	2	5	2	1	7	2	2	4
5.	1709/13	1	1	2	4	1	2	6	7	2	2	4
6.	546/10	3	2	3	2	3	1	1	7	2	2	4
7.	1152/10	2	2	1	3	5	1	1	7	1	1	1
8.	606/13	2	1	2	4	2	1	1	2	2	2	4
9.	5725/6	2	1	5	3	2	1	1	2	2	2	4
10.	1804/11	2	2	6	4	3	1	2	7	2	2	4
11.	521/11	4	1	4	1	1	1	1	7	2	2	4
12.	1986/13	3	1	2	1	2	1	1	1	2	2	4
13.	317/12	2	1	2	4	3	1	1	3	2	2	4
14.	3524/13	1	2	4	4	5	1	1	1	2	2	4
15.	3519/13	2	1	4	2	1	1	1	7	2	2	4
16.	3526/13	3	1	8	4	1	1	1	3	2	2	4
17.	3528/13	2	1	6	4	5	2	1	3	2	2	4
18.	3688/13	2	1	4	4	1	1	6	7	1	1	3
19.	3806/13	1	2	8	4	5	2	3	7	2	2	4
20.	3804/13	2	1	2	4	3	1	3	3	1	1	2
21.	2021/13	3	1	7	3	2	1	2	3	1	1	2
22.	3869/13	2	1	6	4	5	2	2	1	2	2	4
23.	4011/13	2	2	3	1	3	1	1	2	2	2	4
24.	3895/13	2	1	1	4	1	1	1	7	2	2	4
25.	6295/10	2	2	4	1	5	1	2	3	2	2	4
26.	4256/13	2	1	4	4	2	1	1	7	2	2	4
27.	4266/13	3	2	2	2	5	1	3	7	1	1	1
28.	4472/12	2	1	3	4	1	2	5	3	1	1	2
29.	4470/13	3	2	2	2	3	2	2	3	2	2	4
30.	4791/13	1	1	2	4	2	1	1	3	1	1	4
31.	4422/13	2	1	1	4	1	1	1	7	1	1	4
32.	4378/13	2	1	4	4	3	1	1	5	2	2	4
33.	5104/13	3	2	5	3	4	2	2	1	1	2	2
34.	5270/13	2	1	6	1	4	1	2	3	1	2	1
35.	252/07	2	1	5	1	4	1	2	3	1	1	1
36.	230/05	3	2	3	1	5	2	2	2	1	1	2
37.	5400/13	2	2	2	2	3	1	1	7	1	1	2
38.	5405/12	3	1	1	3	5	2	1	1	2	2	4
39.	6894/11	2	1	4	4	1	2	1	7	2	2	4
40.	3148/09	2	1	5	1	3	1	3	5	1	2	2
41.	5506/13	3	1	2	3	3	1	1	7	2	2	4
42.	5001/13	2	1	8	4	2	1	1	3	2	2	4
43.	5440/13	2	1	1	1	5	2	1	7	1	2	3
44.	2894/13	2	1	2	1	1	2	4	5	2	2	4
45.	5524/13	2	2	5	4	5	1	1	3	1	2	2
46.	5527/13	2	2	2	4	5	1	2	7	1	2	2
47.	5549/13	3	2	5	2	3	2	3	7	2	2	4
48.	5517/13	3	1	4	1	5	1	2	3	1	2	2
49.	5578/13	2	1	3	3	2	1	1	2	1	1	2
50.	5939/13	3	1	4	2	2	1	1	7	1	1	1

51.	4388/13	2	1	5	1	3	2	1	7	2	2	4
52.	4169/13	3	1	2	2	1	2	2	6	2	2	4
53.	780/00	4	1	6	4	1	1	1	5	1	2	3
54.	5778/13	4	2	4	1	5	1	4	5	1	1	2
55.	5380/12	1	2	3	4	3	1	4	7	1	2	2
56.	5410/13	2	1	2	2	5	1	4	7	1	1	2
57.	5365/13	2	1	5	2	2	1	6	4	1	2	2
58.	5959/13	2	1	1	4	1	2	5	7	1	2	2
59.	6039/13	2	1	4	3	5	2	1	7	1	1	1
60.	5909/13	2	1	8	4	1	1	2	2	1	1	1
61.	6094/13	3	1	1	4	2	1	1	3	1	1	2
62.	6091/13	4	2	2	1	5	1	4	6	1	2	2
63.	6187/13	2	1	5	4	2	2	1	7	2	2	4
64.	6194/13	1	1	4	4	1	2	1	7	2	2	4
65.	6223/13	2	2	6	1	3	2	1	1	2	2	4
66.	6293/13	2	1	2	4	1	2	4	5	1	1	4
67.	6311/13	2	1	2	4	1	1	4	5	1	1	1
68.	625/11	2	1	4	1	5	2	4	3	1	1	1
69.	611/13	3	2	6	1	5	2	2	1	2	2	4
70.	6325/13	4	2	5	4	5	2	4	2	2	2	4
71.	6335/13	3	2	4	3	5	1	1	3	1	1	2
72.	6219/13	3	1	2	4	2	2	4	2	2	2	4
73.	2755/00	3	2	6	2	3	2	1	2	2	2	4
74.	4833/13	3	1	1	1	2	2	1	1	2	2	4
75.	7343/13	3	1	8	4	5	2	2	1	2	2	4
76.	5845/13	3	1	4	2	2	2	1	7	2	2	4
77.	5474/13	1	2	5	4	5	2	3	2	2	2	4
78.	6509/13	2	1	1	2	1	2	1	1	2	2	4
79.	208/11	3	1	2	1	1	2	3	7	2	2	4
80.	6550/13	2	1	6	4	2	1	5	7	1	1	1
81.	6500/13	4	2	1	3	5	2	2	2	2	2	4
82.	6499/13	2	1	2	2	2	1	4	3	1	1	2
83.	6331/13	2	1	4	1	3	2	1	6	1	2	4
84.	6656/13	4	1	5	3	1	1	2	3	1	1	4
85.	6648/13	4	1	3	1	1	1	3	3	1	1	4
86.	4487/13	3	1	2	1	1	1	1	3	2	2	4
87.	6276/13	2	2	4	4	2	1	1	7	2	2	4
88.	6659/13	4	1	1	1	2	1	1	2	1	1	3
89.	829/13	2	1	6	4	1	2	2	5	2	2	4
90.	6294/13	3	2	2	2	2	1	1	3	2	2	4
91.	6509/13	4	1	4	4	2	2	1	6	2	2	4
92.	6672/13	2	1	4	4	4	2	3	7	2	2	4
93.	6806/13	2	2	6	1	3	1	1	7	2	2	4
94.	6706/13	4	2	2	3	1	2	1	7	2	2	4
95.	3220/13	2	1	4	1	5	1	1	5	2	2	4
96.	8871/13	4	1	5	1	3	2	1	2	2	2	4
97.	6861/13	2	1	2	4	2	2	1	6	2	2	4
98.	6589/13	4	1	5	2	2	2	5	5	2	2	4
99.	6986/13	2	1	4	4	1	2	4	3	1	2	3
100.	6952/13	3	1	5	1	5	2	1	1	2	2	4
101.	6994/13	2	2	1	4	2	2	3	7	2	2	4
102.	5396/13	2	1	4	4	1	2	2	6	2	2	4
103.	6948/13	2	2	4	1	2	1	1	3	1	1	1
104.	7124/13	3	1	2	2	3	2	4	5	2	2	4
105.	7258/13	4	1	5	1	4	2	5	5	2	2	4
106.	7312/13	3	1	2	4	1	2	4	3	2	2	4
107.	7319/13	2	1	1	4	5	2	2	2	2	2	4
108.	7346/13	2	2	1	1	5	1	1	5	1	1	4
109.	7340/13	4	1	2	3	1	2	1	2	2	2	4
110.	7354/13	4	1	4	2	5	2	1	3	2	2	4
111.	7394/13	2	1	2	4	5	2	1	3	2	2	4
112.	3129/10	3	1	4	1	2	1	1	7	1	2	1
113.	7513/13	2	1	5	1	1	2	1	4	1	2	1
114.	7591/13	2	1	2	2	1	1	1	7	1	1	1

115.	1542/13	2	1	1	3	2	2	1	1	2	2	4
116.	7498/13	2	1	4	3	1	2	1	3	2	2	4
117.	7611/13	2	1	1	1	3	2	3	3	1	2	1
118.	6997/12	1	1	4	4	4	2	1	3	2	2	4
119.	7450/13	2	2	2	1	3	2	1	2	2	2	4
120.	7589/13	2	1	1	4	5	2	1	3	1	2	2
121.	7491/13	4	1	6	3	1	1	3	3	1	1	1
122.	8077/11	2	1	7	4	5	2	1	6	2	2	4
123.	7568/13	3	1	2	2	1	2	1	2	2	2	4
124.	7539/13	3	2	3	1	2	2	1	1	2	2	4
125.	45/14	2	1	2	4	5	2	1	5	2	2	4
126.	96/14	3	1	2	1	2	2	1	7	2	2	4
127.	58/14	3	1	4	4	1	2	3	7	2	2	4
128.	104/14	4	1	5	3	1	1	1	2	2	2	4
129.	128/14	2	2	4	2	5	2	1	7	1	1	1
130.	7244/13	3	1	1	2	5	2	2	3	2	2	4
131.	154/14	2	1	2	4	4	2	3	3	2	2	4
132.	197/14	3	2	6	1	5	2	2	3	2	2	4
133.	6996/09	3	1	5	3	1	2	1	7	2	2	4
134.	194/14	2	1	8	4	5	2	1	3	2	2	4
135.	3446/10	2	1	2	4	5	1	4	7	1	2	2
136.	269/14	2	1	4	1	4	1	1	3	1	1	3
137.	302/14	3	2	5	3	1	2	1	2	2	2	4
138.	225/14	4	2	1	3	3	1	2	3	1	2	1
139.	7967/11	2	1	4	4	2	2	1	7	2	2	4
140.	386/14	4	1	2	2	5	1	1	3	1	2	2
141.	361/14	2	2	6	4	5	2	1	5	2	2	4
142.	517/14	3	2	2	1	3	2	2	7	1	2	1
143.	497/11	1	1	4	4	5	2	1	5	2	2	4
144.	3226/03	2	1	4	4	1	1	4	3	1	1	2
145.	7317/13	3	1	2	2	2	1	1	3	1	1	2
146.	7513/13	3	2	5	1	3	2	4	4	1	2	4
147.	7542/13	4	1	4	3	2	1	5	3	1	2	2

Age **clinical features** **past history** **personal history**

<20 years-1 PPF-post prandial fullness-1 DM- diabetes-1 AL-alcohol intake-1
21-39 years -2 BL- abdominal bloating-2 HT- hypertension-2 SM-smoking-2
40-59 years -3 ES -early satiety -3DG- drug intake-3 CO-coffee-3
>60 years -4 EB-epigastric burning-4 NIL-no past history -4 SK-skip meals-4
AN-relived by antacids -5; NO-nil history -5
HB-heart burn -6
NV- nausea &vomiting -7;
CP-atypical chest pain-8

RUT- rapid urease test **HPE- histopathological** **OGD findings**

Positive- 1
Negative- 2

1 = CNG-chronic non -specific gastritis
2 = EG- erosive gastritis
3 = NG- nodular gastritis
4 = IM- intestinal metaplasia
5 = AG-atrophic gastritis
6 = HP- h. pylori

1 = TYPE -1
2 = TYPE-2
3 = TYPE-3
4 = TYPE-4
5 = DU- duodenal ulcer
6 = GU – gastric ulcer
7 = N- normal muocosa

PROFORMA

PROFORMA

NAME : AGE/SEX : DDHD no:

HISTORY:

POSTPRANDIAL FULLNESS: UPPER ABDOMINAL BLOATING:

EARLY SATIETY: EPIGASTRIC BURNING : RELIEVED BY

ANTACIDSBELCHING: WATER BRASH: ODYNOPHAGIA

: NAUSEA: VOMITING:

HEARTBURN:Chest Pain: PURPURA: EASY FATIGUBILITY:

PAST HISTORY

DM: SHT: TB:

IHD : DRUG/ NSAID / ASA INTAKE:

PERSONAL HISTORY:

SMOKING: ALCOHOL : SKIPING OFMEALS :

TOBACCO: CAFFINE: Emotional stress :

Sleep Pattern :

FAMILY HISTORY: Similar illness :

GENERAL EXAMINATION:

CONSCIOUS/ORIENTED: PALLOR: ICTERUS:

CYANOSIS: CLUBBING: EDEMA:

LYMPHADENOPATHY: PURPURA:

OTHER SIGNS:

VITALS: HT: WT: BMI:

PULSE: BP: TEMP: URINE OUTPUT:

SYSTEMIC EXAMINATION:

ORAL CAVITY: P/A :

R.S : CVS: CNS:

INVESTIGATIONS:

Hb : ESR: TC : DC:P L E M

BT: CT: Pl COUNT:

RBS: UREA: CREATININE:

ECG: CHEST X RAY:

USG ABDOMEN:

Date - OGD REPORT:

RUT:

HPE:

C/S: AMOX: METRO: CLARITHROMYCIN:

TETRACYCLINE: LEVOFLOXACIN: BISMUTH:

RIFABUTIN:

PCR:glmm:

cag PAI : Vac A - S1M1 : S1 M2:

INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Ref.No.10499/ME-1/Ethics/2012 Dt:06.12.2012.
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study on Occurrence of virulent strains of helicobacter pyloriand its association with endoscopic findings in a teritiary centre teaching hospital in chennai" for dissertation purpose submitted by Dr.V.Anand, 2nd year DM.,Gastroenterology, PG Student, Kilpauk Medical College Chennai.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

CHAIRMAN, 11/3/14
Ethical Committee
Govt.Kilpauk Medical College,Chennai

GOVT. KILPAUK MEDICAL COLLEGE
11 MAR 2014
Kilpauk, Chennai-10.